



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No.: 09/905,907
Filing Date: 17 July 2001
Inventor(s): Vladimir BARANOV et al.
Title of Invention: **Elemental Analysis of Tagged Biologically Active Materials**

DECLARATION OF SCOTT D. TANNER, PhD.

Scott D. TANNER, PhD., being duly warned that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom, declares that:

1. I am an Associate Professor at the Institute of Biomaterials and Biomedical Engineering at the University of Toronto, in Toronto, Ontario, Canada, and have held that position since March 01, 2005. Prior to that and since January 01, 2001, I was a Principal Research Scientist at MDS Sciex, 71 Four Valley Drive, Concord, Ontario; and prior to that I was a Senior Research Scientist at MDS Sciex since June 01, 1980. I am a joint inventor of the material disclosed in United States patent application 09/905,907 entitled *Elemental Analysis of Tagged Biologically Active Materials* and filed 17 July 2001 (the "Application"). I have more than 29 years of experience in the field of mass spectrometry, primarily in the area(s) of Inductively Coupled Plasma Mass Spectrometry, Corona Discharge Triple Quadrupole Mass Spectrometry, and Townsend (Glow) Discharge Triple Quadrupole Mass Spectrometry. My experience includes extensive research and publishing in the area of mass spectrometry. Details of my experience in this area, including my publications, are set out in my C.V., a copy of which is attached to this affidavit as Exhibit A.

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2. The Application is directed to the analysis of analytes bound to biologically active affinity materials, for example, antigens, antibodies, peptides, etc, by tagging such biologically active materials with a transition element, and detecting the tagged biologically active materials using an inductively coupled plasma mass spectrometer (ICP-MS). In one aspect, the invention may be viewed as involving the combination of two disciplines: (i) ICP-MS and (ii) the tagging of biologically active affinity materials. A particular advantage of the invention is that it provides a greatly increased multiplexing capability, which allows the analysis of many analytes in one sample.

3. ICP-MS was first described, more than 20 years prior to the filing of the Application, in a paper published by R.S. Houk, V.A. Fassel, G.D. Flesch, H.V. Svec, A.L. Gray and C.E. Taylor, *Analytical Chemistry* 52, 2283-2289 (1980). A copy of the Houk et. al article is attached hereto as Exhibit B.

4. The earliest reference of which I am aware to the tagging of biologically active materials is E. Frieden, M.B. Lipsett and R.J. Winzler, *Science* 107, 353-354 (1948), which describes radiometric tagging. The earliest reference to fluorometric tagging that I am aware of is K.E. Osserman and L.B. Weiner, *Annals Of The New York Academy Of Sciences* 124, 730 (1965). A copy of the Frieden reference is attached hereto as Exhibit C.

5. The use of ICP-MS in the tagging of biologically active materials was first described by me and my joint inventors in the Application, which was filed in the United States Patent and Trademark Office (the "Office") on 17 July 2001.

6. In rejecting claims of the Application, the Office cited U.S. patent 4,205,952 to Cais, Specific Binding Assay Method and Reagent Means ("Cais"). Cais discloses a method of tagging materials with elements, including transition elements, with the expressed object of providing a specific binding assay method for the determination of small amounts of unknown compounds. Cais neither discloses nor suggests any use of inductively coupled plasma

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generators (ICPGs) or ICP-MS systems in the tagging of any biologically active materials, or in multiplexing, or in any particular types of mass spectrographic analysis whatever, or provides any motivation for such use.

7. In rejecting claims of the Application the Office further cited U.S. patent 6,242,735 to Li et al., Power-Modulated Inductively Coupled Mass Spectrometry ("Li"). Li discloses techniques for power modulation in inductively coupled plasma generators (ICPGs). Li neither discloses nor suggests any use of ICPGs or ICP-MS systems in the tagging of any biologically active materials, or in multiplexing, or in any particular types of mass spectrographic analysis whatever, or provides any motivation for such use. Li is concerned solely with engineering issues related to the reduction of power consumption in ICPGs by controlling heat-loading by the ICP on the mass spectrometer, so as to reduce the cooling requirements and thus the cost of the ICP-MS instrument.

8. In rejecting claims the Office further cited Schramel, P, (CANAS '95, Colloquium Analytische Atomspektroskopie, Konstanz, Germany, April 2 - 7, 1995 (1996), 671-681 "Schramel". Schramel discloses the comparative analysis of untagged biological materials (for example, animal tissue and blood) using an optical emission spectrometer (OES) and a mass spectrometer. Schramel concludes that OES can detect a number of mineral and trace elements which is difficult for MS, whereas an advantage of MS lies in the determination of heavy metals. Schramel neither discloses nor suggests the tagging of any biologically active materials, or provides any motivation for such use.

9. The need or desirability of a highly-multiplexed method of detecting analytes, and efforts made to solve that need, may be inferred from the earliest publications in flow cytometry. The earliest specific reference to multiplex assay of analytes that I am aware of is P.K. Horan and L.L. Wheelless Jr, *Science* 198, 149-157 (1977) (attached hereto as Exhibit D). This paper cites the following papers that apparently discuss multiparametric assays,: L.A. Herzenberg, R.G. Sweet and L.A. Herzenberg, *Scientific American* 234, p108 (1976); J.A. Steinkamp, M.J. Fulwyler, J.R. Coulter, R.D. Hiebert, J.L. Horney and P.F. Mullaney, *Reviews of Scientific*

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Instruments 44 p1301 (1973); J.A. Steinkamp and H.A. Crissman, *J. Histochem. Cytochem.* 22 p616 (1974); B.J. Fowlkes, C.J. Herman and M. Cassidy, *J. Histochem. Cytochem.* 24 p322 (1976); M. Stohr, *Pulse Cytophotometry* 2 p39 (1976); R. Curbelo, E.R. Schildkraut, T. Hirschfeld, R.H. Webb, M.J. Bolck and H.M. Shapiro, *J. Histochem. Cytochem.* 24 p388 (1976); and H.M. Shapiro, E.R. Schildkraut, R. Corbelo, R.B. Turner, R.H. Webb, D.C. Brown and M.J. Block, *J. Histochem. Cytochem.* 25 p836 (1977). In particular, the last paper refers to a 3-laser system, each capable of 5 parameter detection and suitable for 7-parameter assay. A somewhat later paper that specifically addresses an N-plex assay is T.N. Buican and G.W. Hoffmann, *Cell Biophysics* 7, 129-156 (1985). Efforts to successfully implement such a method have been sought continuously since. The need for detecting numerous analytes has included, for example, detecting (i) markers on cancer cells, (ii) toxins in environmental samples, (iii) endogenous proteins in cells, (iv) exogenous proteins in cultured cells following transfection, for example as reporter assays, (v) elemental species, (vi) detecting receptors in drug discovery assays, (vii) detecting ischemic markers in patients believed to have suffered a heart attack.

10. The need for a highly-multiplexed method of detecting analytes has persisted, and if anything has grown more compelling over the years. The need has been intensified, for example, due to the ongoing discovery of numerous biological markers in cells, the increased use of transfection in biomedical research, the increased testing of environmental samples, and increased drug discovery research.

11. Although:

- (a) the need for a highly multiplexed method of detecting analytes has persisted since at least 1977;
- (b) tagging of biologically active materials has been known since at least 1948; and
- (c) the ICP-MS was first described in 1980,

no one thought of, or succeeded in, until myself and my co-inventors Vladimir BARANOV, Dmitry BANDURA and Zoë QUINN conceived and reduced to practice the invention disclosed in the Application, combining ICP-MS with tagging of biologically active affinity materials in order to fill

such needs. Thus those needs went unfulfilled, and the advantages provided by the systems and methods disclosed in the Application were not available, for at least 20 years, despite the existence of components required for the solutions disclosed in the Application.

12. Others have attempted, but failed, to provide systems and methods capable of highly-multiplexed detection of biologically active and affinity materials using a variety of approaches. For example, scientists have tried to solve this need using fluorescent flow cytometry. Fluorescent flow cytometry has the ability to perform multiplex assay, typically up to 4-plex. More recent work, especially in the Herzenberg laboratory at Stanford University, has been directed at increasing the degree of multiplex capability, where up to 12-plex assays are conceivable by fluorescence under certain stringent conditions. Such efforts are described, for example, in the attached Exhibits H - J (see below). In fact, the explosive field of microarrays has arisen specifically to address the need for multiplexing: since only a few fluorophores are distinguishable, the microarrays address the issue by providing a matrix of assay spots that provide two additional degrees of multiplex. As a further example, the development of "multicolored" beads by Luminex was also directed at this challenge: beads are labeled with different concentrations of two fluorophores, the ratio of which distinguishes the beads from each other, so that a multiplex of the order of 100 is achievable. However, fluorescent flow cytometry is prone to signal overlap between fluorophores and the number of biologically active or affinity materials that can be simultaneously assayed by any of the alternative approaches is not equal to that enabled through the use of ICP-MS. Thus the need has not been solved by others.

13. The invention has been praised as an important and innovative step by numerous independent members of the scientific community. For example, the following are among comments received by the inventors from the International Scientific Review Committee of the Genome Canada Applied Health Grant Competition, 2003 (regarding related work concerning instrumentation and reagents used in implementing the method described in the patent application, applied to single cells in a flow cytometer configuration) and evidenced by materials attached hereto in Exhibit E:

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- "... the impact of successful completion of the proposed research project would be huge."
- "This is a very exciting and highly innovative proposal..."
- "The project is innovative and likely to have high scientific and potentially high future clinical impact."
- "By any measure this is a remarkable proposal. The proposed studies will develop a novel and highly sophisticated technology to address an essential issue in cancer biology."
- "...potential to revolutionize the way in which cells are analyzed."
- "This proposal represents a very strong and innovative combination of talents and expertise from disparate but highly complementary research groups."
- "The proposed work addresses a novel strategy to bring flow cytometry an entirely new level, capable of increasing sensitivity and increasing multiplexing capacity."
- "The proposal is very innovative and original."
- "The beauty of the newly proposed instrument is that it is a combination of two techniques that previously were used by very different disciplines but which together provide exciting new capabilities."
- "The project describes an innovative use of ICP-MS to create a multiplexed detector that will have a wide variety of uses."
- "Finally, the expertise in ICP-MS in Dr Tanner's laboratory at MDS-Sciex, together with his recent developments in its use for biopolymer analysis, open the way to increased specificity and sensitivity of detection of tagged cells. Thus, one of the most appealing aspects of the current proposal is the combination of expertise from previously disparate fields, namely the application of ICP-MS, a tool previously used primarily for inorganic elemental analysis, to research in cell and molecular biology. In a sense, this conceptual leap has already been made by the MDS Sciex members of the present group of applicants, who recently described quantitative element-tagged immunoassay with ICP-MS detection. The present proposal is a novel extension of this approach, incorporating very important additional ideas."

As a further example, the following are among comments received by the inventors from the United States National Institute of Health (NIH) grant review committee, 2005 (regarding related work concerning development of specific tags described generically in the patent application), as shown in materials attached hereto in Exhibit F:

- "Strengths noted were the highly innovative aspects of the approach, the potential impact of being able to achieve multiplexed analyses..."
- "The proposal is highly innovative in proposing a new type of tag that can reasonably achieve a high level of multiplexing."
- "It would be highly advantageous to perform immunoassays in a multiplexed fashion,

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where a complex mixture of selective antibodies can identify, for example, an unknown pathogen, rather than testing the pathogen with many different antibodies.”

“This is a very interesting proposal that could in fact have very significant repercussions in the field of clinical prognostic assays if it is successful.”

“The proposed research could potentially have an enormous impact on immunoassays, bring down the cost and speed of medical testing, and likely saving lives by identifying pathogens faster.”

“The ability to provide clinicians and researchers with a better immunoassay that can be used in a multiplex fashion, such as ICP-MS, is very attractive and could have profound ramifications in the medical community.”

“The proposed plan is quite innovative as there are very few, if any, others who have conducted the prescribed tagging methods.”

“There is significant potential for widely-useful assays to result from this work, and for widespread use of these methods in real clinical environments.”

14. Since the first public disclosure of the invention disclosed in the Application, the invention has attracted significant attention among other researchers and has spawned a large amount of further research. The first public disclosure of our invention was made in February of 2001 in *ICP-MS as an Elemental Detector In Immunoassays. Speciation Without Chromatography*, V.I. Baranov, D.R. Bandura and S.D. Tanner, European Winter Conference on Plasma Spectrochemistry, Hafjell, Norway, Winter 2001, Book of Abstracts, p 85, a copy of which is attached hereto as Exhibit G. Our first three papers on element-tagged immunoassay were published in 2002. Copies of those papers are attached hereto as Exhibits H, I, and J respectively:

A Sensitive and Quantitative Element-Tagged Immunoassay with ICP-MS Detection, V.I. Baranov, Z. Quinn, D.R. Bandura and S.D. Tanner, Analytical Chemistry, **74**,1629 (2002).

Simultaneous Determination of Cell Lysate Proteins at Endogenous Levels using Element-Tagged Immunoassay Coupled with ICP-MS Detection, Z.A. Quinn, V.I. Baranov, S.D. Tanner, and J.L. Wrana, Journal of Analytical Atomic Spectrometry, **17**, 892 (2002).

The Potential for Elemental Analysis in Biotechnology, V.I. Baranov, Z.A. Quinn D.R. Bandura and S.D. Tanner, Journal of Analytical Atomic Spectrometry, **17**, 1148

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(2002).

These papers have been cited by at least 19 other papers, which in turn have been cited by numerous other researchers. Citations of our papers describing the invention are included in (copies attached as Exhibits K - Y respectively):

Trace element speciation by ICP-MS in large biomolecules and its potential for proteomics, Sanz-Medel A, Montes-Bayon M, Saanchez MLF, Analytical and Bioanalytical Chemistry 377 (2): 236-247 2003. This paper has been cited at least a further 23 times, including by Hogbom, Ballihaut, Lobinski, Szpunar, Fernandes, Miquel, Sanz-Medel, Liu, Waddell, Li, Atanassova, Bindila, Ray, St Remy, Matsunaga.

Structural identification and quantification of protein phosphorylations after gel electrophoretic separation using Fourier transform ion cyclotron resonance mass spectrometry and laser ablation inductively coupled plasma mass spectrometry, Becker JS, Boulyga SF, Becker JS, et al., International Journal of Mass Spectrometry 228 (2-3): 985-997 15 2003. This paper has been cited at least a further 17 times, including by Gomez-Ariza, Becker, Feldmann, Gunther, Szpunar, Marshall, Ray, McLeod, Bai, Broekaert, Jakubowski, Lehmann.

Determination of phosphorus and metals in human brain ablation inductively coupled plasma source mass spectrometry, Becker JS, Zoriy M, Becker JS, et al., Journal of Analytical Atomic Spectrometry 19 (1):149-152 2004. This paper has been cited at least a further 15 times, including by Becker, Lobinski, Feldmann, Schaumlöffel, Taylor, Szpunar, Fisher, Marshall, Ray, McLeod, Marchante-Gayon.

Determination of phosphorus in small amounts of protein samples by ICP-MS, Becker JS, Boulyga SF, Pickhardt C, et al., Analytical and Bioanalytical Chemistry 375 (4): 561-566 2003. This paper has been cited at least a further 11 times, including by Gomez-Ariza, Becker, Szpunar, Caruso, Kruger, Broekaert, Jakubowski.

Element and molecular mass spectrometry - an emerging analytical dream team in the life sciences, Wind M, Lehmann WD, Journal of Analytical Atomic Spectrometry 19 (1):20-25 2004. This paper has been cited at least a further 11 times, including by Ballihaut, Lobinski, Feldmann, Szpunar, Jensen, Ray, Becker, Kruger.

Atomic spectrometry update. Clinical and biological materials, foods and beverages, Taylor A, Branch S, Halls D, et al., Journal of Analytical Atomic Spectrometry 18 (4):385-427 2003. This paper has been cited at least a further 10 times, including by Hieftje, Liu, Hight, Potts, Beauchemin, Engstrom, Niemela, Taylor, Cubadda

Reaction cell inductively coupled plasma mass spectrometry based immunoassay using ferrocene tethered hydroxysuccinimide ester as label for the determination of 2,4-dichlorophenoxyacetic acid, Deng AP, Liu HT, Jiang SJ, et al., Analytica Chimica Acta 472 (1-2): 55-61 2002. This paper has been cited at least a further 5 times, including by Ueng, Zhang, Jiang.

Metallobiomolecules. The basis of life, the challenge of atomic spectroscopy, Jakubowski N, Lobinski R, Moens L, Journal of Analytical Atomic Spectrometry 19 (1): 1-4 2004. This paper has been cited at least a further 4 times, including by Hogbom, Fisher, Atanassova, Engstrom.

Speciation of protein-bound trace elements by gel electrophoresis and atomic spectrometry, Ma RL, McLeod CW, Tomlinson K, et al., Electrophoresis 25 (15): 2469-2477 2004. This paper has been cited at least a further 3 times, including by Ballihaut, Feldmann, Szpunar.

Sensitive time-resolved fluoroimmunoassay for simultaneous detection of serum thyroid-stimulating hormone and total thyroxine with Eu and Sm as labels, Wu FB, Han SQ, Xu T, et al., Analytical Biochemistry 314 (1): 87-96 2003. This paper has been cited at least a further 2, times including by Pan, Zhang.

Advances in analytical methodology for bioinorganic speciation analysis: metallomics, metalloproteomics and heteroatom-tagged proteomics and metabolomics, Szpunar J, Analyst, 130 (4): 442-465 2005. This paper has been cited at least a

metalloproteomics and heteroatom-tagged proteomics and metabolomics, Szpunar J, Analyst, **130** (4): 442-465 2005. This paper has been cited at least a further 2 times, including by Sperling and Ballihaut.


Simultaneous determination of alpha-fetoprotein and free beta-human chorionic gonadotropin by element-tagged immunoassay with detection by inductively coupled plasma mass Spectrometry, Zhang SC, Zhang C, Xing Z, et al., Clinical Chemistry 50 (7): 1214-1221 2004.

ICP-MS-based competitive immunoassay for the determination of total thyroxin in human serum, Zhang C, Wu FB, Zhang XR, Journal of Analytical Atomic Spectrometry 17 (10): 1304-1307 2002.

Characterization of a gadolinium-tagged modular contrast agent by element and molecular mass spectrometry, Kruger R, Braun K, Pipkorn R, et al., Journal of Analytical Atomic Spectrometry 19 (7):852-857 2004.

15. The facts set forth in this Declaration are true, and all statements made of my own knowledge are true, and all statements made on information and belief are believed to be true.

Dated: 21-OCTOBER-2005

By: 
Scott D. TANNER, PhD.